Detection of Leukocyte Activation in Pigs with Neurologic Decompression Sickness

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NYQUIST PA, DICK EJ, BUTTOLPH TB. *Detection of leukocyte activation in pigs with neurologic decompression sickness.* **Aviat Space Environ Med 2004; 75:211–4.**

Background: In a porcine model of neurological decompression sickness (DCS), perivascular leukocyte activation was a consistent finding in biopsies of associated cutaneous DCS. This prompted examination of other organs for similar changes; multifocal leukocyte activation was found in the lungs (pneumonitis) and liver (hepatitis). ***Hypothesis:*** DCS in pigs induces leukocyte aggregation and activation in the liver and lungs. ***Methods:*** Male Yorkshire swine, trained to run on a modified treadmill, were compressed to 200 ft of seawater (fsw) in a dry, air-filled compression chamber. Decompression varied according to the profile under study. ***Results:*** In 106 pigs, evidence for association of leukocyte aggregation and activation with the clinical diagnosis of neurologic DCS was sought. The incidence of pneumonitis (20/68, 29% with DCS; 4/38, 10% without DCS) and hepatitis (23/68, 33% with DCS; 4/38, 10% without DCS) were strongly correlated with the incidence of neurologic DCS via Pearson Chi-squared analysis ($p = 0.026$ pneumonitis and $p = 0.008$ hepatitis). Additionally, Kruskal-Wallis rank analysis for numbers of organs involved and incidence of neurologic DCS showed a strong correlation between the increasing occurrence of neurologic DCS and the involvement of both the liver and lungs ($p = 0.004$). ***Conclusions:*** The results imply that, at least in pigs, DCS induces leukocyte aggregation and activation in the liver and lungs. These organs are not normally considered targets of DCS. Leukocyte aggregation in these organs may be related to their roles as highly perfused organs. Leukocyte aggregation may be a marker for DCS, providing further evidence for wider, systemic effects of DCS.

Keywords: decompression sickness, organ perfusion, leukocyte aggregation, neutrophils.

A PIG MODEL OF decompression sickness (DCS) has been developed that closely resembles the clinical and pathological features of severe human DCS (2). Study of cutaneous DCS, which also occurs in the model (5), has shown perivascular leukocyte activation suggestive of an inflammatory process, even in the absence of morphological tissue damage. We sought similar histopathologic changes in tissues theoretically exposed to relatively high bubble counts as a consequence of their high level of perfusion. The organs examined include the lungs and liver. We have observed neutrophil infiltration in DCS of the skin, and neutrophils may be markers of bubble exposure in other organs. By looking for neutrophils in other organ systems in animals with decompression sickness, we could look for changes in organs not normally considered to be affected by decompression stress.

METHODS

All procedures were conducted in accordance with National Research Council guidelines on laboratory animal use. Before commencing, the Institutional Animal Care and Use Committee reviewed and approved this protocol. The institutional animal care facility is fully AALAC accredited.

Animal Model

The model is described in detail elsewhere (2) and only a summary is provided here. Juvenile, male Yorkshire swine (weight range 15–25 kg), were trained to run on a modified laboratory treadmill. Each pig completed a simulated dive to 200 ft of seawater (fsw) in a dry, air-filled compression chamber. Compression took 5 min: 2 min at a rate of 20 fsw $\cdot$ min^{-1} to a pressure of 40 fsw, 1 min at 40 fsw $\cdot$ min^{-1} to 80 fsw, then 60 fsw $\cdot$ min^{-1}.

On returning to the surface, pigs were removed from the chamber and observed for developing signs of neurological DCS. They were then sedated and treated by recompression on a standard U.S. Navy Treatment Table VI. If, after 1 h, no subjective neurological signs were observed, the pigs ran on the treadmill and their gait was assessed. Pigs with no discernable gait abnormality at 1 h and 24 h post dive were categorized as functionally unaffected.

Sample Collection and Histopathology

After functional assessment at the 24-h evaluation, pigs were anesthetized by i.v. injection of ketamine, 400 mg, and xylazine, 20 mg, then euthanized by cardioplegia with bolus i.v. injection of 40 ml 4-molar potassium chloride solution. Two liters of 0.9% saline, followed by

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This manuscript was received for review in June 2003. It was revised in August and September 2003. It was accepted for publication in September 2003.

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Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE **2004**	2. REPORT TYPE **N/A**	3. DATES COVERED **-**

4. TITLE AND SUBTITLE **Detection of Leukocyte Activation in Pigs With Neurologic Decompression Sickness**	5a. CONTRACT NUMBER 5b. GRANT NUMBER 5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER 5e. TASK NUMBER 5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Naval Medical Research Center Silver Spring, MD 20910**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S) 11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**SAR**	**4**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

TABLE I. DECOMPRESSION SICKNESS VERSUS HEPATITIS AND PNEUMONITIS.

	No DCS	DCS	Total
Hepatitis*			
Hepatitis	4	23	27
No Hepatitis	34	45	79
Total	38	68	
Pneumonitis†			
Pneumonitis	4	20	24
No Pneumonitis	34	48	82
Total	38	68	

* $p = 0.0$; † $p = 0.026$.

4 L of 10% paraformaldehyde, were immediately infused by peristaltic pump via a common carotid artery. Both femoral veins were incised to provide free drainage of blood infused fluid.

Following perfusion fixation, a complete necropsy was performed. The heart was examined to exclude patent foramen ovale. A complete set of tissues was collected and placed in 10% neutral buffered formalin. After 3 d fixation, tissues were trimmed, embedded in paraffin, sectioned at 6 pm, stained with hematoxylin and eosin, and evaluated by light microscopy. The veterinary pathologist was blind to the incidence of DCS in each sample. Only the liver and the lungs were chosen for analysis because they were the organs available in significant numbers with a predominant venous blood flow for examination.

Statistical Analysis

A Pearson Chi-square test of 2 × 2 contingency tables was used to compare the incidence of organ involvement with the incidence of neurologic DCS. All statistics were calculated using SYSTAT for Windows, taking $p < 0.05$ as the threshold of statistical significance (9). The Kruskal-Wallis test technique (9) was used to test for a relationship between the number of organs involved and the incidence of DCS. The rankings used were as follows: no lesion (0), either hepatitis or pneumonitis (1), both hepatitis and pneumonitis (2). Although a lesser number of animal subjects would have sufficed for this experiment, the results were obtained from data collected from a previous study on neurological DCS that required 124 subjects to achieve the desired power.

RESULTS

Of 124 pigs initially examined, 18 were found to have bronchopneumonia when their lungs were examined histologically. These pigs were excluded from the analysis. The specimens from the remaining 106 pigs were included in the study.

Of the 106 pigs, 68 developed neurologic DCS and 38 were functionally unaffected. The distribution of pneumonitis and hepatitis is shown in **Table I**. Pneumonitis and hepatitis were significantly associated with a clinical diagnosis of neurologic DCS.

Characteristic CNS lesions produced by the model are described in detail elsewhere (5), but the most common findings outside of the nervous system included acute to subacute pneumonitis, hepatitis, and skin bends. The pneumonitis was characteristically composed of small aggregates of neutrophils surrounding the vasculature within the parenchyma, with occasional macrophage and rare lymphocytes infiltrating and transmigrating the septal wall and within the lumen of the alveoli (**Fig. 1**). The hepatitis was comprised of similar aggregates of inflammatory cells typically within, and minimally distending, hepatic sinusoids. Neutrophil infiltration into the adjacent hepatic parenchyma and hepatocyte necrosis was frequently observed (**Fig. 2**).

To test the hypothesis that multiple organ involvement was more highly associated with DCS than no organ involvement, each pig was ranked according to the number of organs involved (lung or liver involvement vs. involvement of both organs). There were 62 pigs with no lung or liver involvement, 37 with either lung or liver involvement, and 7 with both organs involved (**Table II**). A Kruskal-Wallis one-way analysis of variance for 106 cases was completed. A substantial and significant relationship was detected between increasing incidence of neurologic DCS and the number of

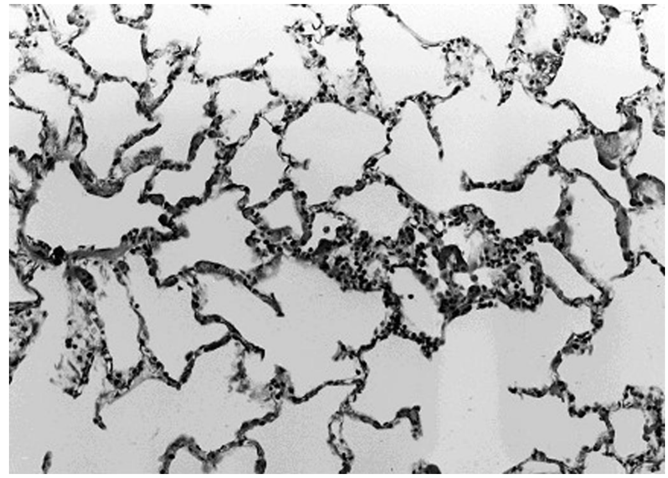

Fig. 1. Light microscopy of lung tissue; small aggregates of neutrophils can be seen surrounding the vasculature.

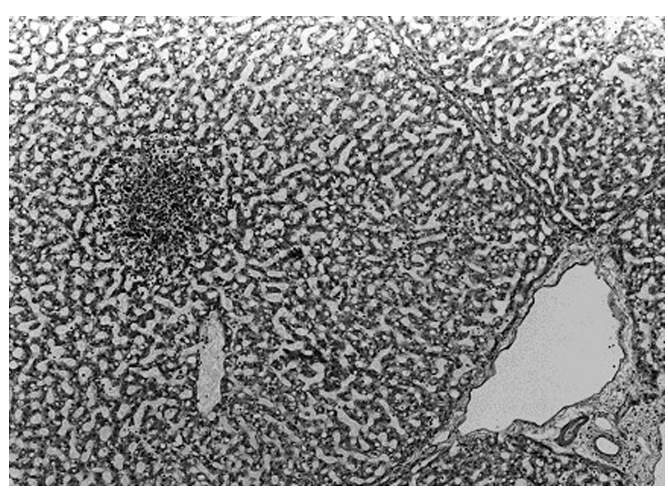

Fig. 2. Light microscopy of liver specimens with leukocyte aggregation.

TABLE II. KRUSKAL-WALLIS RANK SUM OF MULTIPLE ORGAN INVOLVEMENT.

Group	Count	Rank Sum
None Involved	62	2905.0
One Involved	37	2258.5
Two Involved	7	507.5

Test Statistic = 11.299.
p Value = 0.004.

organs involved ($p = 0.004$, and a Kruskal-Wallis test statistic of 11.299).

DISCUSSION

By using leukocytes as markers of DCS, we have detected evidence of this malady in the liver and lungs. Two conclusions from this study are suggested: 1) DCS is a multisystem disease involving many organ systems at a subclinical level, and 2) leukocyte activation and aggregation is related to DCS and can be used as a marker for DCS.

Electron micrographic studies of skin biopsy specimens from pigs with skin DCS showed sequential adhesion and migration of leukocytes across cutaneous venular endothelium, consistent with an early vasculitis (5). Organs were available from a series of pigs that participated in a previous study (2). This study reviewed the histologic findings from these specimens and found a strong association between the clinical diagnosis of neurologic DCS and acute inflammatory changes in other organs. The predominance of neutrophils on histological examination suggests that these findings are an acute, as opposed to chronic, process. This suggests that the phenomena occurred after diving and not before.

All statistical testing demonstrated p-values well below 0.05 significance, with findings that are substantial as well as statistically significant. Two independent statistical methodologies support the same conclusion: the Kruskal-Wallis and the Pearson Chi-squared. The Kruskal-Wallis analysis also shows a relationship between increasing numbers of organs involved and decompression stress. This finding is consistent with our assertion that increasing decompression stress results in a greater number of organs with subclinical signs of decompression stress. These facts argue against the case of statistical epiphenomena.

Etiology of Leukocyte Activation in Lungs and Liver

The aggregation of leukocytes in the lung and liver of the pigs in this study could result from one of two primary mechanisms: 1) an activation of leukocytes in the targeted organs resulting from increased bubbles filtering through the lungs and liver, which are highly perfused organs; or 2) perivascular infiltration of leukocytes in these highly perfused organs as a consequence of filtering activated leukocytes from the general circulation in the liver and lungs.

In both cases leukocyte activation could result from: 1) an ischemic stimulus from a distant or local site; 2) reaction to local mechanical tissue damage due to bubbles; or 3) a systemic, chemically mediated response to intravascular inert gas bubbles. Local nonischemic leukocyte activation could result from damage to the endothelium by bubbles of inert gas. In all scenarios, endothelial cells or hematogenous white cells would subsequently release mediators such as cytokines, complement, and leukotreines, which would then function to activate and attract leukocytes (1,6–8,13–16,21–24,26).

In this study, it is not clear which mechanism resulted in the aggregation of leukocytes in the lungs and liver. All the pigs that dove sustained significant decompression stress. In light of this, it is not surprising that some pigs demonstrated systemic signs of leukocyte activation in the absence of neurologic symptoms of DCS.

The most likely stimulus for leukocyte activation is the influence of inert gas bubbles on the local endothelium of the tissues involved (7,15,16). This stimulus is probably physical disruption of the endothelium resulting in transient local ischemia or mechanical activation of the endothelium. This theory is partially supported by our histologic findings of local tissue necrosis often associated with the vasculitis. Leukocyte aggregation is probably not occurring as a result of the filtration of activated leukocytes from the bloodstream. Hepatitis and/or pneumonitis was found in some animals with neurologic, but no other histologic, signs of DCS. Leukocyte accumulation in other highly vascularized organs, such as the kidneys, should also have been found if this was simple filtration of activated leukocytes from a distal source. These findings indicate that damage to endothelial cells is occurring locally with subsequent leukocyte infiltration.

The incidence of hepatitis is higher than pneumonitis, yet the lung is more highly perfused than the liver. It also receives a greater percentage of the blood flow than the liver. Increased hepatic involvement may be expected for three reasons. First, the liver filters much of the venous circulation prior to transit to the lungs and serves as a prefilter before the larger initial bubble loads transit to the lungs. Second, bubble formation is primarily a venous (low pressure) phenomena; the liver receives a substantial portion of its blood supply from low-pressure venous systems (1,6,13,14,20,25). Third, the abdomen and liver have been associated with large boluses of bubbles during decompression (3,17). Increased involvement of the liver may be the result of large bubble loads arriving directly from intestinal sources into the hepatic circulation.

Multisystem Involvement of DCS

Acute DCS is manifested as disturbances of the central nervous system (CNS), joints, and skin; chronic DCS primarily affects bone. The symptomatology of DCS has been associated with bubble formation in the blood of animals and humans affected by the disease. Many reports of organ involvement at sites other than the joints or the CNS exist. Examples include reports of cardiac (Ball R, personal communication, 1996), ocular (4), and hepatic involvement (3). Our data suggest extensive histological evidence of involvement in the liver and lungs at a subclinical level.

At present, a unifying theory has been lacking to explain many observations about the variety of clinical manifestations of DCS. Our observations would support a hypothesis that DCS may have systemic effects that result from vascular endothelium damaged by gas bubbles. This damage may provoke an inflammatory or immune response resulting in leukocyte activation (6–8,15,16).

Hyperbaric oxygen, the primary modality for the treatment of DCS (5,11,18,23,25), has been shown to inhibit leukocyte aggregation (15,16,24,26). The association between DCS in pigs and leukocyte aggregation suggests that hyperbaric oxygen may have an anti-inflammatory effect possibly mediated by decreased white cell aggregation. The perception of DCS as an inflammatory phenomenon responsive to hyperbaric oxygen is very attractive. It would explain many of the clinical peculiarities of DCS, such as the late response of DCS to hyperbaric oxygen therapy, acclimatization (19,24), individual susceptibility (Thalmann ED, Flynn ET, personal communication, 1995), the similarity of symptoms of DCS to symptoms of rheumatic diseases, and anecdotal reports of the ability of hyperbaric oxygen to relieve the symptoms of many immune-mediated diseases as well as DCS (10,12).

CONCLUSIONS

This study offers histologic evidence that DCS is a multisystem disease involving the liver as well as the lungs. It also offers evidence that DCS results in white cell aggregation and that white cell aggregation can act as a marker of DCS.

ACKNOWLEDGMENTS

This work was supported by the Naval Medical Research and Development Command, Work Unit No. 637 13N M0099.O1C-1610. The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official at large.

The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, DHHS, Publication No. (NIH) 86–23 (1985).

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